THIN-FILM INTRACORTICAL RECORDING MICROELECTRODES

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by the

Center for Wireless Integrated MicroSystems

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Thin-Film Intracortical Recording Microelectrodes

Summary

During the past quarter, we fabricated our ninth custom mask set of passive probes for a variety of users, including many in the Neural Prosthesis Program. These included probes for use in spinal cord, for use in recording from cat auditory nerve, and for recording and stimulating rat cortex. Some of these included 3D probes to be assembled using polymer cables attached to the probes using rivet bonds. The polyimide cables are now in fabrication at Michigan. A 16-site chronic probe was also fabricated that includes back-side wells designed to hold gels seeded with a neurotrophin such as NGF. We hope to get experience with these probes in-vivo during the coming term.

We have also been histologically investigating the tissue sheath that forms around the probes in-vivo. Vimentin immunoreactive cells were found in the region of the probe tract at three days following placement, forming a rim around the tract by one week following placement. This suggests that meningeal epithelial cells are either pushed down during probe placement or rapidly migrate down the probe shank. While some glial fibrillary acidic protein (an intermediate filament marker for reactive glia - astrocytes) immunoreactive cells were seen at three days, they were first seen to make a major contribution to the envelope of cells around the probe at one week. This contribution increased over the time of placement, with a larger contribution visible at 3 weeks following placement. The contribution from vimentin immunoreactive cells could be seen to make up the inner layer of the envelope and GFAP IR cells a more outer layer. A neuronal contribution to the tissue envelope is less consistent. Neurofilament immunostained processes can be seen in the tissue envelope; however, this occurs in less than 50% of the probe shanks assessed and also varies along the length of the probe shank.

We have designed a number of new active recording probes. The first of these is a 32-channel probe designed for recording from hippocampus in freely-moving rats. This probe features amplifiers with a programmable lower cutoff frequency, suitable for recording both single units and field potentials. An 8-site probe for simultaneous stimulation and recording has also been designed along with a 12-site chronic probe for use in rat motor cortex. A six-channel active probe has also been designed to allow a direct comparison between noise with and without on-chip amplification. Finally, a final iteration of our 64-site multiplexed active probe with front-end site selection (Pia-2) has been designed. The noise of the on-probe amplifiers has been reduced from 16.6µVrms to 7.1µVrms and low frequency cutoff tuning has been included on some of the probes. The use of our active probes in Dr. Gyorgy Buzsaki's laboratory at Rutgers is continuing. Chronic probes having active buffers are driving 8 feet of shielded cable while eliminating the need for headstage amplifiers.

Work has also gone forward with the development of a wireless interface for these probes. A 10b successive approximation charge-redistribution analog-to-digital converter has been developed along with a Manchester decoder for taking the neural signals and readying them for transmission to the outside world. At a 4MHz clock, the ADC has a speed of 250kSamples/sec with 10b resolution. Power dissipation totals less than 1.5mW for this data conversion and encoding chip. We hope to operate a recording probe wirelessly during the coming term.

Thin-Film Intracortical Recording Microelectrodes

1. Introduction

The goal of this program is the realization of batch-fabricated recording electrode arrays capable of accurately sampling single-unit neural activity throughout of volume of cortical tissue on a chronic basis. Such arrays will constitute an important advance in instrumentation for the study of information processing in neural structures and should be valuable for a number of next-generation closed-loop neural prostheses, where stimuli must be conditioned on the response of the physiological system.

The approach taken in this research involves the use of solid-state process technology to realize probes in which a precisely-etched silicon substrate supports an array of thin-film conductors insulated above and below by deposited dielectrics. Openings in the dielectrics, produced using photolithography, form recording sites which permit recording from single neurons on a highly-selective basis. The fabrication processes for both passive and active (containing signal-processing circuitry) probe structures have been reported in the past along with scaling limits and the results of numerous acute experiments using passive probes in animals. In moving to chronic implant applications, the major problems are associated with the preserving the viability of the sites in-vivo (preventing tissue encapsulation of the sites) and with the probe output leads, both in terms of their number and their insulation. The probe must float in the tissue with minimal tethering forces, limiting the number of leads to a few at most. The encapsulation of these leads must offer adequate protection for the megohm impedance levels of the sites while maintaining mechanical lead flexibility.

Our solution to the lead problem has involved two steps. The first has been to embed circuitry in the probe substrate to amplify and buffer the signals and to multiplex them onto a common output line. Using this approach, signal levels are increased by factors of over 100, impedance levels are reduced by three to four orders of magnitude, and the probe requires only a few leads for operation, independent of the number of recording sites. A high-yield merged process permitting the integration of CMOS circuitry on the probe has been developed, and this circuitry has been designed and characterized. The second step has involved the development of silicon-based ribbon cables, realized using the same probe technology, to conduct the neural signals to the outside world. These cables have shown significant advantages over discrete leads, both in terms of the ease with which chronic implants can be assembled and in terms of the ability of the cables to survive long-term biased soaks in saline. The cables can be built directly into the probes so that they come off of the wafer as a single unit, requiring no joining or bonding operations between them. The cables are also significantly more flexible than previously-used discrete wire interconnects.

This contract calls for the development of active probes for neural recording. A 64-site 8-channel probe with site selection and signal buffering but no multiplexing has

been developed (PIA-2B) along with a high-end multiplexed probe that includes gain (PIA-2). These probes are now being refined and applied to in-vivo applications. Investigations are on-going to better understand site encapsulation, which limits the lifetime of chronic recording structures, and telemetry is being developed to allow the probes to be operated over a wireless link, eliminating the percutaneous connector.

During the past quarter, we have realized a number of new passive probe designs and have explored the tissue sheath that forms around the probes in-vivo. We have designed a number of new active probes, including a new version of PIA-2/-3, and have realized a new successive approximation data converter and Manchester encoder for use in a wireless link for the probes. Work in these areas is discussed in the sections below.

2. Passive Probe Developments

The Center for Neural Communication Technology fabricated its ninth custom probe mask set, CNCT6, during the last quarter. This mask set includes 25 designs for 12 investigators, some of whom are funded under Neural Prosthesis Program contracts. A subset of these new designs will be shown here.

Doug McCreery and his colleagues at the Huntington Medical Research Institute (HMRI) submitted two designs for their work in cat spinal cord. These designs are the result of several iterations of probes on previous mask sets. One of these designs is shown in Fig. 1. The sharp tips and tapering shanks facilitate insertion into the tough spinal cord. HMRI assembles these probes into a 3-D structure using molded silicone.

The probe shown in Fig. 2 was submitted by Charles Miller's group at the University of Iowa for recording from cat auditory nerve. This design is a second iteration and includes smaller sites ($132\mu m^2$ as opposed to $625\mu m^2$) in an effort to improve spatial selectivity of recordings. This probe also has very sharp tips to penetrate nerve.

The chronic probes shown in Fig. 3 were designed by Daryl Kipke's group at the University of Michigan for recording from and stimulation of rat cortex. The probes have integrated silicon cables to form the interconnect to a percutaneous connector. These probes each have 32 sites distributed across eight shanks.

A family of cortical probes and the corresponding components to make them into 3D assemblies was also designed for Dr. Kipke's group. As shown in Fig. 4, these probes are intended to mate to a polymer cable that takes the signals to a percutaneous connector. A polymer cable, as compared to the standard silicon cable, will add strength and length to the system. Electrical connection between the probes and cables will be formed using "rivet" bonds as described by Meyer, et al. (*IEEE Trans. Advanced Packaging*, 24:366-374, 2001). Polyimide cables are currently in process in the UM SSEL.

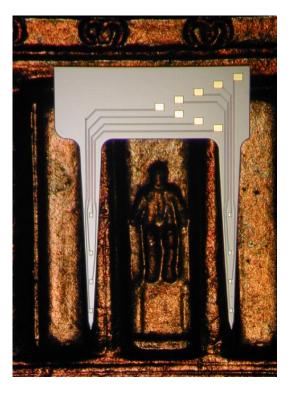


Fig. 1: A probe fabricated for HMRI for spinal cord stimulation shown on the backside of a penny. Multiple probes will be used to assemble a 3-D structure using molded silicone. The shanks are spaced at 1.5mm.

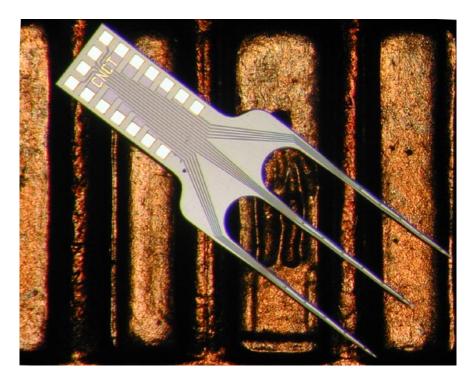


Fig. 2: Probe fabricated for University of Iowa for recording from the auditory nerve of cat. The shanks are spaced at $500\mu m$.

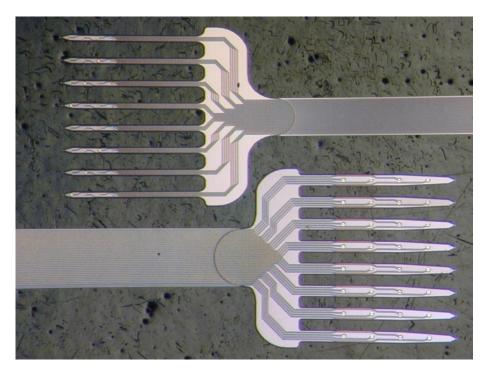


Fig. 3: Chronic probes with integrated silicon ribbon cables for recording (top) and stimulation (bottom) of the rat cortex. Each probe has 32 sites. Shanks are spaced at $200\mu m$.

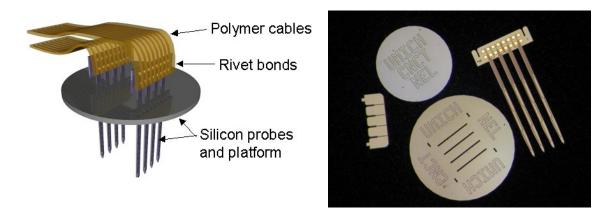


Fig. 4: Hybrid 3-D passive assembly. As shown on the left, silicon probes will be bonded to polymer cables and then inserted into a silicon platform to form a 3-D structure. The silicon components are shown on the right (clockwise from left: spacer to keep probes parallel, handling tab to be placed on top of assembled probes, probe, platform). The platform shown has a diameter of 2.5mm.

The probe shown in Fig. 5 is a 16-site chronic probe designed by David Anderson of the University of Michigan. Although not shown in the photograph, this probe has an

integrated silicon ribbon cable. The design is unique in that it has sites that are exposed from both the front and the back-side of the probe. The back-side of each site is essentially a well that can be used for the local delivery of chemicals to the brain. For example, a gel placed in the well can be seeded with a neurotrophin such as NGF. The sites also have holes through them to permit access to the gel from both sides.

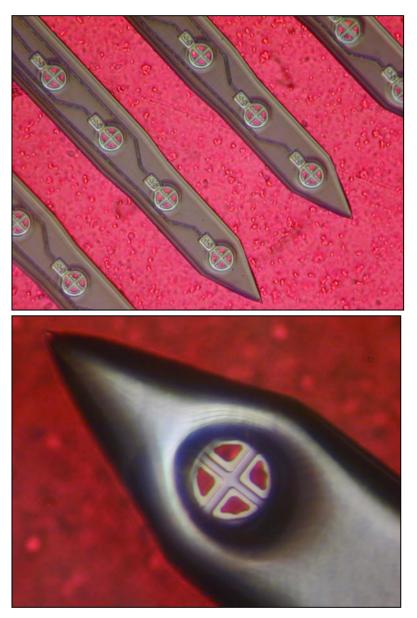


Fig. 5: Front (above) and back (below) sides of a new chronic probe with double-sided sites. This wells on the back-side of each site can be used for local chemical delivery using gel seeded with neurotrophins or other pharmaceuticals. Shanks are spaced at 150µm.

3. Tissue Reactions to Chronic Probes

In conjunction with the Center for Neural Communication Technology (CNCT), we have been using immunocytochemical methods to differentiate the components of the cellular envelope surrounding the electrode tracts in chronically implanted guinea pigs. These stains differentiate and characterize the cellular elements of the tissue envelope that forms to encapsulate each shank of the electrode. We have used the following test group of antibodies:

Intermediate Filaments

Neurofilament 200 - Neurons and neuronal processes GFAP - Glial cells (greatest in reactive glia - astrocytes)

Cytokeratin - Epithelial cells

Vimentin - Epithelial cells of mesenchymal origin

Desmin - Muscle

Collagen - Epithelial

Fibronectin - Extracellular matrix, epithelial

Laminin - Extracellular matrix - blood vessel walls, neuronal

MAB328 - Labels oligodendrocytes and CNS myelin

OX-42 - labels monocytes, granulocytes and macrophages

Recently, we have been using these antibodies to examine how the elements of the tissue envelope change over time of placement. Vimentin is an intermediate filament found in epithelial cells of mesenchymal origin. Vimentin immunoreactive (IR) cells were found in the region of the tract at three days following placement (Fig. 6), forming a rim around the tract by one week following placement (Fig. 7). This suggests that meningeal epithelial cells are either pushed down during probe placement or rapidly migrate down the probe shank.

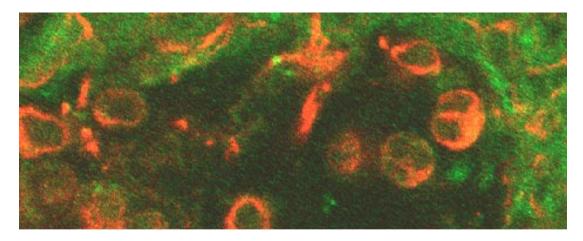


Fig. 6: Co-Labeling for Vimentin (red) and Glial FIbrillary Acidic Protein (GFAP) (Green) Immunoreactivity (IR). Many Vimentin IR cells are seen around the probe tract 3 days following electrode placement in the auditory cortex, while only minor GFAP immunolabeling is found.

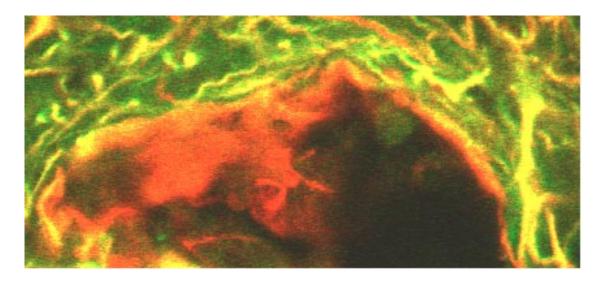


Fig. 7: Co-Labeling for Vimentin (red) and GFAP (green) IR one week following electrode placement in the auditory cortex. The innermost layer is made up of Vimentin IR cells with a small rim of GFAP cells making up a second layer.

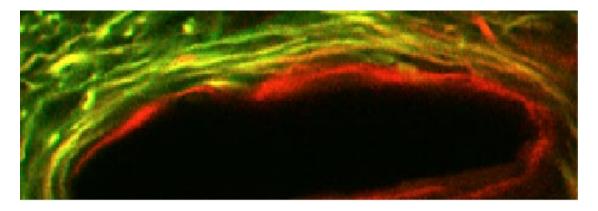


Fig. 8: Co-labeling for Vimentin (red) and GFAP (green) IR, three weeks following electrode placement in the auditory cortex. The outer rim of GFAP IR cells has increased over what is found at 1 week. The black area shows where one of the removed electrode shanks was located, its dimension is $50\mu m \times 15\mu m$.

While some glial fibrillary acidic protein (an intermediate filament marker for reactive glia - astrocytes) IR cells were seen at three days, they were first seen to make a major contribution to the envelope of cells around the probe at one week, seen with a green label in Fig. 7. This contribution increased over the time of placement, with a larger contribution visible (again with green label) in Fig. 8 at 3 weeks following placement. With co-labeling for GFAP (green) and Vimentin (red) the contribution from vimentin IR cells could be seen to make up the inner layer of the envelope and GFAP IR cells a more outer layer.

A neuronal contribution to the tissue envelope is less consistent. Neurofilament immunostained processes can be seen in the tissue envelope (Fig. 9); however, this occurs in less than 50% of the probe shanks assessed and also varies along the length of the probe shank.

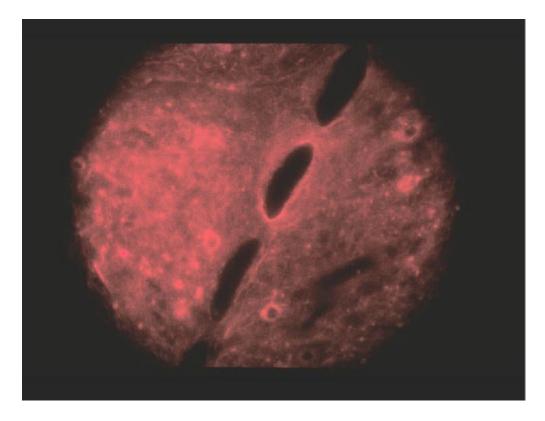


Fig. 9: Neurofilament stain showing an array of shank penetrations in tissue.

Immunostaining for the extracellular matrix protein, fibronectin, is often seen in association with the probe tract. This is likely a product of the meningeal fibroblasts making up the most inner layer. In some cases (Fig. 10) it forms a "path" suggesting there was movement of the probe through the tissue.

These results show that the cellular response to chronic placements of the electrodes is highly consistent with the general wound healing literature. The first response is epithelial cells, most likely of meningeal origin, forming the innermost rim of the cellular envelope. The next component to form, making up the outer portion of the cellular envelope, is glial, with this component increasing over three weeks of placement. The presence of neuronal components are inconsistent. It will be interesting to compare and contrast the size and components of the tissue envelope for electrode sites with different performance characteristics.

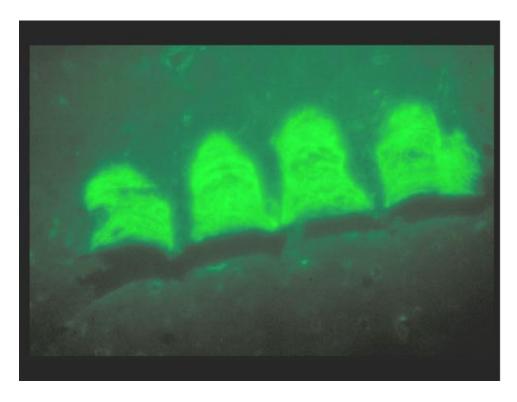


Fig. 10: Fibronectin stain of an array of shank penetrations in tissue.

4. Design and Fabrication of Active Recording Probes

In preparation for an active neural probe fabrication run, which is currently underway, six new neural recording probes with on-chip circuitry have been designed. The first probe, layout pictured in Fig. 11, is a 32 channel electrode designed in conjunction with Gyorgy Buzsaki's Lab at Rutgers to record from the hippocampus of free moving rats. The on-probe circuitry, Fig. 13, features 32 amplifiers and four 8:1 time-division multiplexers to reduce the number of interconnects coming off of the electrode. Because this probe needs to record both field and unit activity, the on-probe amplifiers have been designed with a tunable low frequency cutoff. Including on-probe amplification should further reduce the motion artifacts associated with recording from free moving animals.

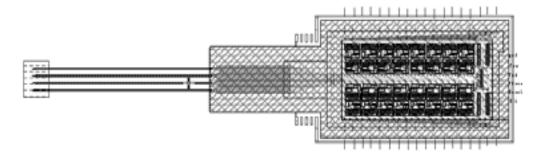


Fig. 11: A thirty-two channel probe for recording in hippocampus of freely-moving rats

The layout of an 8-site electrode for simultaneous stimulation and recording in the hippocampus of free-moving mice is shown in Fig. 12. The on-probe circuitry features 8 pre-amplifiers that can be bypassed in order to directly stimulate each of the 8 sites, Fig. 14. Including CMOS switches to provide access to the site only when stimulating prevents capacitive loading of the site while recording. As with the 32 channel probe for recording in rats, the amplifiers on this probe also have a tunable low frequency cutoff for recording both field and unit activity.

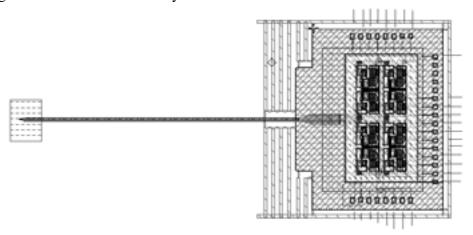


Fig. 12: Eight channel probe for simultaneous recording and stimulation in the hippocampus of freely-moving mice.

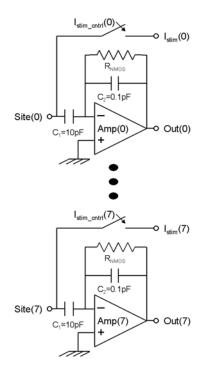


Fig. 14: Block diagram of on-probe circuitry for the probe shown in Fig. 12.

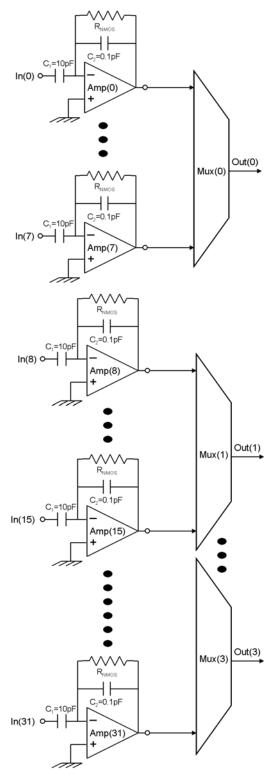


Fig. 13: Block diagram of the on-probe circuitry for the probe in Fig. 11.

The layout of a 12-site chronic probe for evaluating the performance of active electrodes in the motor cortex of the rat is shown in Fig. 15. The probe features 12 on-

chip amplifiers with a gain of 40dB and *in vivo* testing capabilities. The shank and bond pad configurations are identical to the new CNCT chronic rat probes which allows a polyimide cable to be attached to the probe as shown in Fig. 16. Adding active circuitry to the existing CNCT probes will make assembling and implanting the active chronic probes easier and will allow for a more direct comparison between active and passive neural arrays.

A 6-channel active probe for chronic implant studies in the motor cortex of the rat is currently being fabricated with the layout shown in Fig. 17. This probe uses the shank and bond pad configurations incorporated on the CNCT chronic passive electrodes in order to simplify the chronic assembly. The probe features six on-probe tunable amplifiers that can be bypassed to record from the sites directly, Fig. 18. This probe will allow direct comparison of signal to noise ratio of passive and active recordings. Particularly of interest will be the SNR of the active and passive channels over time, where deterioration of the ribbon cable dielectrics will degrade the SNR of the passive channels before that of the active channels due to the low output impedance of the amplifiers. This probe can also be used to compare the SNR of active and passive channels when recording outside of a shielded room, which will be necessary when implementing a fully implantable closed-loop neural prosthesis.

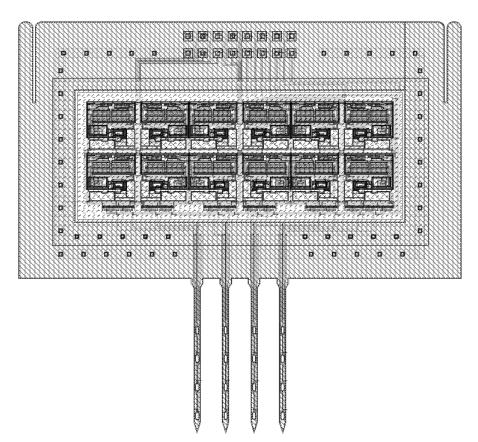


Fig. 15: Twelve-site chronic electrode designed for recording in rat motor cortex

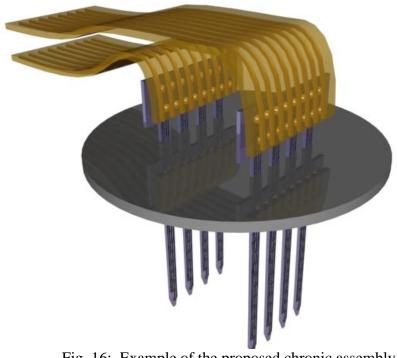


Fig. 16: Example of the proposed chronic assembly

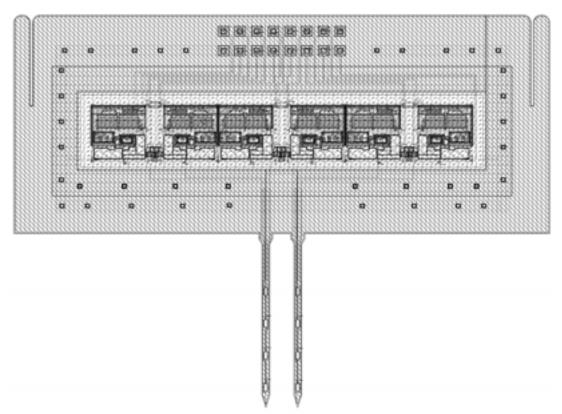


Fig. 17: Six-site chronic probe designed to compare SNR of passive and active recordings

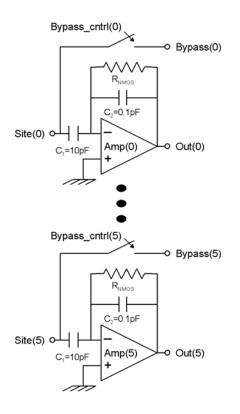


Figure 8. Block diagram of the on-chip circuitry for the probe shown in Fig. 7

A third chronic probe has been designed to evaluate the chronic performance of the LTO and plated gold shield used to package the circuit areas of the probe. A layout of this probe is shown in Fig. 19. The probe has one amplified channel, two test resistors, and six test transistors.

A 64 site, eight channel active electrode with on-probe front-end selection, amplification and time-division multiplexing is currently being fabricated with a layout pictured in Fig. 20 and a block diagram of the on-probe circuitry shown in Fig. 21. This is the second iteration of the PIA-2 probe that was successfully characterized in the spring of 2002. The noise of the on-probe amplifiers has been reduced from 16.6µVrms to 7.1µVrms and low frequency cutoff tuning has been included on some of the probes. The arrays have been designed with both gold beam leads and bond pads at the back of the probes to allow for both acute and 3D assemblies.

A three week chronic experiment in Gyorgy Buzsaki's Lab at Rutgers using a 64 site probe implanted in the hippocampus of a free moving rat has shown that motion artifacts can be reduced through the use of on-probe buffers. The response of identical passive and active probes to a tap on the micro-manipulator used to position the implant is shown in Fig. 22. Oscillations resulting from the tap of the micro-manipulator can be clearly seen on the passive channels while the buffered channels continue to record neural potentials. The response of a single passive (Fig. 23) and active (Fig. 24) channel

to a tap of the micro-manipulator is shown below. After a tap of the micro-manipulator only motion artifacts can be seen on the passive channel while unit activity can be recorded using the buffered electrodes.

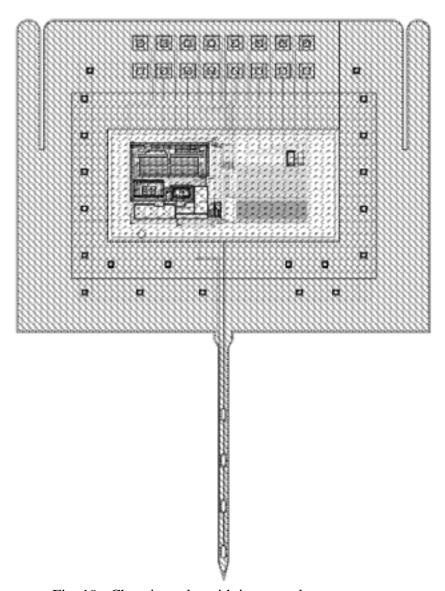


Fig. 19: Chronic probe with integrated test structures

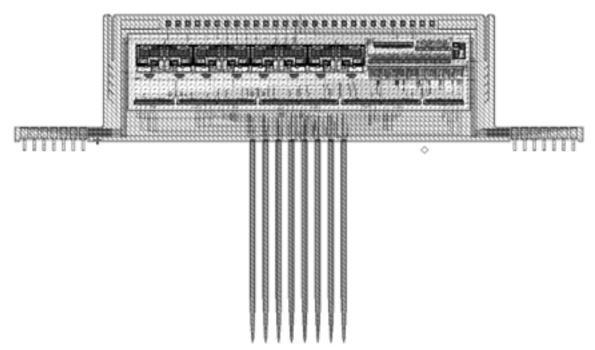


Fig. 20: A 64 site, 8 channel, chronic neural recording array with on-probe front-end selection, amplification and time-division multiplexing circuitry

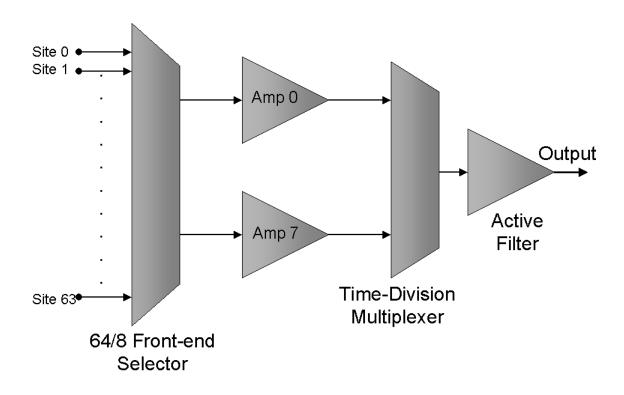


Fig. 21: Block diagram of the on-probe circuitry for the probe in Fig. 20

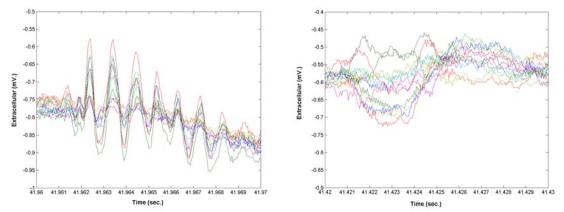


Fig. 22: Response of twelve channels of an otherwise identical passive (left) and active (right) neural probe to a single tap of the micro-manipulator.

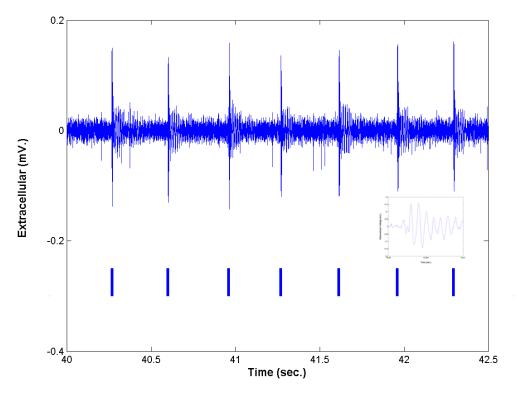


Fig. 23: Single channel passive probe response to tapping of micro-manipulator. Solid bars indicate timing of tapping. Inset shows spike detail.

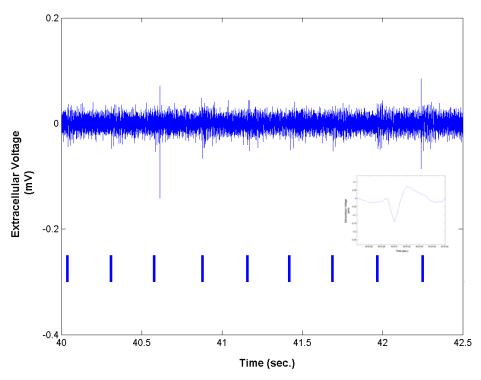


Fig. 24: Single channel active probe response to tapping of micro-manipulator. Solid bars indicate timing of tapping. Inset shows spike detail.

5. Design of a Wireless Telemetry Platform for Multichannel Microprobes

During the past quarter, an ADC chip was fabricated which includes a 10-bit hybrid successive approximation charge-redistribution ADC used in time-division multichannel recording along with some logic circuitry. The chip is being tested and some of measurement results are shown in this report.

ADC chip

A fabricated ADC chip shown in Fig. 25 includes a hybrid charge-redistribution 10-bit ADC used in time-division multi-channel recording, and some logic circuitry. A block diagram of the ADC circuitry is shown in Fig. 26. An input analog signal is fed to the 10-bit ADC, and the 10-bit digital result is stored in a 16-bit shift register, where a 6-bit header is added for data recognition. A Manchester encoder then encodes serial bits to synchronize data transmission.

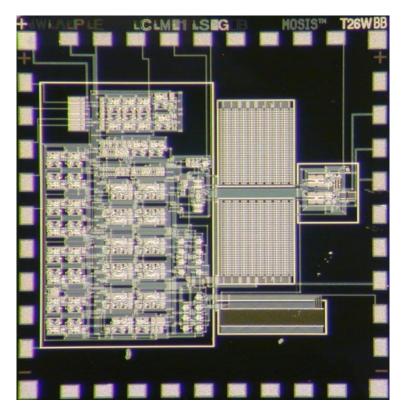


Fig. 25: The fabricated ADC chip

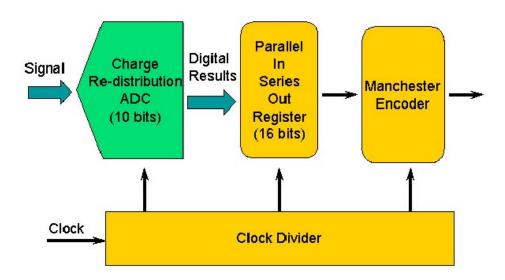


Fig. 26: Block diagram of the circuitry on the ADC chip

The functionality of clock divider and ADC is verified and shown in Fig. 27.



Fig. 27: The results for clock divider and ADC

In Fig. 27, channel 1 is the input 8MHz clock, which is divided by two and from which the 4MHz clock, shown in channel 2, is generated. It takes 16 cycles of the 4MHz clock for the ADC to finish A-to-D conversion for one sample, so the speed is 250kS/s as shown above. When the ADC finishes the conversion, a data ready signal, shown in channel 3, is generated to latch 10-bit digital data into a 16-bit parallel-in-serial-out shift register. The result of 1000000001 for a 1.65V input voltage is shown in Channel 4 and confirms correct operation of the ADC. The clock is 4MHz and the time for one A-D conversion is 4µs. More A-to-D conversions for input voltages of 0V and 3.3V are shown in Fig. 28 and Fig. 29. ADC linearity is shown in Fig. 30. Some logic circuits are also included on the chip.

The 10-bit ADC parallel output is latched into a shift register, a test header "101110" is added, and then the output is fed to a Manchester encoder. The waveforms shown in the above figures confirm the functionality of this logic block. The Manchester encoder is used to encode the digital output of the ADC for synchronization in data transmission. The description of Manchester encoding can be found in previous reports, and this circuit block works as expected, as shown in Fig. 31. Bit "1" from the shift register triggers a rising edge at the output during the succeeding clock period, while Bit "0" causes a falling edge at the output during the succeeding clock period. The measurement results are summarized in Table 1.



Fig. 28: ADC operation for input of 0.0V



Fig. 29: ADC operation for input of 3.30V

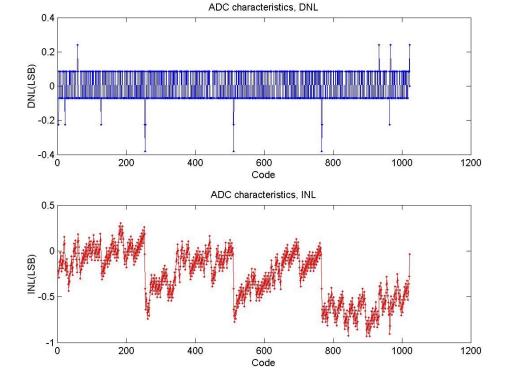


Fig. 30: ADC Linearity



Fig. 30: Manchester Encoding

Circuit Blocks	Specification	
	Clock Used	4MHz / 2MHz
10-bit Charge Re-distribution	Speed	250kS/s / 125kS/s
ADC	Resolution	10 bit
	DNL/INL	DNL <0.4LSB,
	(Fig. 6)	INL <0.9LSB
Manchester Encoder	Encoding data before transmission for synchronization	
Parallel-in-series-out 16-bit Shift Register	"Data Ready" signal from ADC latches 10bit results into this register, add 6-bit header.	
Clock Divider	Generate 4MHz, 2MHz, 1MHz, 500kHz, 250kHz, 125kHz clocks for on-chip circuitry	
	Analog Part	185.5μW
Power dissipation (At 4MHz)	Digital Part	1225μW
	Total	1.41mW
Power dissipation (At 2 MHz)	Analog Part	159.7μW
	Digital Part	601µW
	Total	761µW

Table 1: Summary of the measurement results of ADC chip

6. Conclusions

During the past quarter, we fabricated our ninth custom mask set of passive probes for a variety of users, including many in the Neural Prosthesis Program. These included probes for use in spinal cord, for use in recording from cat auditory nerve, and for recording and stimulating rat cortex. Some of these included 3D probes to be assembled using polymer cables attached to the probes using rivet bonds. The polyimide cables are now in fabrication at Michigan. A 16-site chronic probe was also fabricated that includes back-side wells designed to hold gels seeded with a neurotrophin such as NGF. We hope to get experience with these probes in-vivo during the coming term.

We have also been histologically investigating the tissue sheath that forms around the probes in-vivo. Vimentin immunoreactive cells were found in the region of the probe tract at three days following placement, forming a rim around the tract by one week following placement. This suggests that meningeal epithelial cells are either pushed down during probe placement or rapidly migrate down the probe shank. While some glial fibrillary acidic protein (an intermediate filament marker for reactive glia - astrocytes) immunoreactive cells were seen at three days, they were first seen to make a major contribution to the envelope of cells around the probe at one week. This contribution increased over the time of placement, with a larger contribution visible at 3 weeks following placement. The contribution from vimentin immunoreactive cells could be seen to make up the inner layer of the envelope and GFAP IR cells a more outer layer. A

neuronal contribution to the tissue envelope is less consistent. Neurofilament immunostained processes can be seen in the tissue envelope; however, this occurs in less than 50% of the probe shanks assessed and also varies along the length of the probe shank.

We have designed a number of new active recording probes. The first of these is a 32-channel probe designed for recording from hippocampus in freely-moving rats. This probe features amplifiers with a programmable lower cutoff frequency, suitable for recording both single units and field potentials. An 8-site probe for simultaneous stimulation and recording has also been designed along with a 12-site chronic probe for use in rat motor cortex. A six-channel active probe has also been designed to allow a direct comparison between noise with and without on-chip amplification. Finally, a final iteration of our 64-site multiplexed active probe with front-end site selection (Pia-2) has been designed. The noise of the on-probe amplifiers has been reduced from 16.6µVrms to 7.1µVrms and low frequency cutoff tuning has been included on some of the probes. The use of our active probes in Dr. Gyorgy Buzsaki's laboratory at Rutgers is continuing. Chronic probes having active buffers are driving 8 feet of shielded cable while eliminating the need for headstage amplifiers.

Work has also gone forward with the development of a wireless interface for these probes. A 10b successive approximation charge-redistribution analog-to-digital converter has been developed along with a Manchester decoder for taking the neural signals and readying them for transmission to the outside world. At a 4MHz clock, the ADC has a speed of 250kSamples/sec with 10b resolution. Power dissipation totals less than 1.5mW for this data conversion and encoding chip. We hope to operate a recording probe wirelessly during the coming term.